cultured under varying concentrations of leptin alone, adiponectin alone or together with the pro-inflammatory cytokines TNF-α and IL-1β. Lactate was used to assess energy metabolism. Active and proMMP-2 and -9 in the culture medium were measured using gelatin zymography. Western blotting was used to assess levels of MMP-1, -3 and -13 and quantitative real-time PCR was used to assess expression levels of anabolic and catabolic genes.
Results: Leptin influenced cellular metabolism of both the NP and OA cells. Furthermore leptin led to decreased glycosaminoglycan production and significantly increased production of MMP-2, -3 and -9, by both cell types at the protein level. Similar results were seen with adiponectin. Gene expression of the matrix genes aggrecan, collagen I and collagen II fell with increasing concentrations of leptin. Most importantly, in the presence of leptin, the expression of both TNF-α and IL-1β were significantly upregulated. Addition of leptin to medium containing the pro-inflammatory cytokines, demonstrated a marked synergistic effect on energy metabolism and the production of certain proteases, especially MMP-2.
Conclusions: Our results show that leptin can upregulate proteases involved in degenerative processes in the IVD, and that this effect is potentiated in the presence of pro-inflammatory cytokines such as TNF-α and IL-1β. Leptin levels are increased markedly in obese patients and hence a biochemical mechanism may be involved in the association between obesity, disc degeneration and back pain particularly in an inflammatory environment

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OSTEOGENIC DIFFERENTIATION OF IVD CELLS: INDUCTION OF BIOLOGICAL FUSION.

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Purpose: Much focus has been placed on the development of methods for biological repair or regeneration of the degenerate intervertebral disc (IVD), with varying success in humans and animal models. The current 'gold standard' treatment for IVD degeneration is fusion of the vertebral space, requiring invasive and painful surgery with extensive metalwork. Unlike IVD regeneration, little emphasis has been placed on the development of biological fusion. We have investigated the ability for IVD cells to differentiate osteogenically and aim to determine the 'best' method for bone formation. Ideally, this will result in the development of a method to induce the degenerate (and painful) IVD to turn itself to bone and fuse the vertebral space and thus remove the need for major surgery for the patient.
Methods: Monolayer cultures of IVD cells and mesenchymal stromal cells (MSCs) isolated from samples obtained from patients undergoing routine surgery for IVD disorders were treated with standard culture media, osteogenic media (including 100 nM dexamethasone, 10 nM β-glycerophosphate and 50 μM L-ascorbic acid-2-phosphate) or 1,25 dihydroxyvitamin D3 (VitD3) at 0.1, 1 or 10 nM for 21 days. Treated cells were histochemically stained for alkaline phosphatase activity, a marker of osteogenic differentiation.
Results: VitD3 produced a dose-related increased production of alkaline phosphatase by both MSCs and IVD cells, compared to standard culture media. However, the response to osteogenic media was greater. Generally, the response of IVD cells under all culture conditions was less than seen with MSCs, although the trends were always the same.
Conclusions: These preliminary results show that VitD3 can induce osteogenic differentiation of IVD cells, but often to a lesser extent than media containing dexamethasone, β-glycerophosphate and L-ascorbic acid-2-phosphate or that seen from MSCs. This is encouraging in the search for a method to induce biological fusion of the vertebral space, but further work using other osteogenic factors is required.

Joint Morphology and/or Morphometry

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STUDY OF CARTILAGE DAMAGE INDEX WITH JOINT SPACE NARROWING AND KELLGREN-LAWRENCE GRADE

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Purpose: While cartilage morphometry on magnetic resonance (MR) imaging is increasingly accepted as an outcome measure for clinical trials among individuals with osteoarthritis (OA), it remains burdensome, which limits its utility in large studies. We recently developed a rapid knee cartilage quantification method that has been validated with joint space width, joint space narrowing, static alignment. As another step in our validation process we wanted to confirm the cartilage damage index (CDI) can also detect a previously described association between cartilage damage and Kellgren-Lawrence (KL) grade with the exception of KL = 2, which has cartilage thickening.
Methods: We selected 102 participants from the Osteoarthritis Initiative (OAI) who had a diverse range of JSN (0 to 3) and KL grades (0 to 4) and for whom 3D double-echo steady-state sagittal images were available. MRIs were obtained on four 3-Tesla systems (0.37 mm × 0.37 mm, 0.7 mm slice thickness). One reader used customized software to measure the CDI in the medial femur and tibia from the baseline and 24-month visit (ICCs3,1 = 0.95 to 0.99). Central readers determined semi-quantitative assessments of radiographic knee OA severity (KL grade and modified OARSI-atlas based medial JSN scores) using the weight-bearing posterior-anterior fixed-flexion knee radiographs from the baseline OAI visit (radiographic readings are publicly available at http://oai.epi-ucsf.org/; kxr_sq_bu_00 [version 0.5]). To account for different skeletal

Cartilage damage index stratified by baseline medial JSN grade

Cartilage Measure	JSN = 0(n = 43) mean (SD)	JSN = 1(n = 25) mean (SD)	JSN = 2(n = 26) mean (SD)	JSN = 3(n = 3) mean (SD)	p-value for Trend
Femur CDI (Baseline)	747.95 (143.69)	664.55 (98.89)	521.57 (108.73)	308.95 (171.85)	<0.01
Femur CDI (Baseline)	389.56 (78.60)	368.12 (59.16)	292.77 (71.57)	149.53 (113.88)	<0.01
Tibiofemoral CDI (Baseline)	1137.50 (203.22)	1032.70 (149.94)	814.34 (152.48)	458.48 (278.78)	<0.01
Femur CDI (Change)	−2.95 (48.95)	−19.33 (57.29)	−47.15 (60.92)	−99.30 (90.47)	<0.01
Tibia CDI (Change)	−9.31 (26.30)	−26.25 (26.41)	−45.00 (41.63)	−43.86 (44.65)	<0.01
Tibiofemoral CDI (Change)	−12.26 (63.30)	−45.58 (71.29)	−92.15 (82.51)	−143.2 (134.52)	<0.01

Cartilage damage index stratified by baseline KL grade

Cartilage Measure	KL = 0 (n = 4) mean (SD)	KL = 1 (n = 19) Mean (SD)	KL = 2 (n = 40) mean (SD)	KL = 3 (n = 34) mean (SD)	KL = 4 (n = 5) mean(SD)	p-value for Trend
Femur CDI (Baseline)	676.99 (90.01)	657.45 (151.46)	712.84 (117.43)	599.35 (169.07)	527.67 (332.25)	0.02
Tibia CDI (Baseline)	416.01 (97.53)	350.20 (58.16)	378.33 (67.55)	330.49 (94.90)	248.59 (169.46)	<0.01
Tibiofemoral CDI (Baseline)	1093.0 (179.14)	1007.7 (192.65)	1091.2 (170.23)	929.83 (248.63)	776.26 (497.49)	0.01
Femur CDI (Change)	−38.28 (57.02)	−15.08 (52.16)	−4.95 (52.98)	−40.33 (58.57)	−38.70 (107.60)	0.15
Tibia CDI (Change)	−43.46 (50.24)	−18.96 (27.01)	−14.33 (27.37)	−36.19 (38.95)	−36.19 (38.95)	0.23
Tibiofemoral CDI (Change)	−81.74 (106.83)	−34.04 (58.88)	−19.29 (70.13)	−76.52 (79.70)	−65.35 (143.93)	0.12